

Pressure Transducer Method for Measuring Gas Production by Microorganisms

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A simple method for measuring gas production by microorganisms by using a pressure transducer to sense pressure buildup was developed and tested with members of the coliform group. The test system consisted of a 5.0 lb/in² pressure transducer and a pressure equalizer valve attached to the metal cap of a test tube (20 by 150 mm); gas pressure was recorded on a strip-chart recorder. Gas pressure response curves consisted of (i) a lag period with no marked increase in pressure, (ii) a rapid pressure buildup period, and (iii) a leveling-off period. A linear relationship was established between inoculum size and length of the lag period. Cultures shaken at 200 oscillations/min showed a marked increase in rate of gas release over stationary cultures. Cell concentrations at the time of rapid buildup in pressure were 10⁸/ml. Mean maximum pressure recordings, lb/in² per 10 ml of broth, were: *Enterobacter aerogenes*, 3.70; *Citrobacter intermedium*, 2.70; and *Escherichia coli*, 2.10. Mean CO₂ concentrations, ppm of headspace gas, for *E. coli* were: (i) 2,000 at time of inoculation, (ii) 25,000 at time of rapid buildup in pressure, and (iii) 150,000 at maximum pressure. These results indicate the potential application of the pressure transducer method for rapidly detecting coliforms and other gas-producing microorganisms in clinical samples and in sterility testing of foods.

A number of techniques are available for measuring gas production by microorganisms. Recent publications described a radiometric technique for detecting bacteria in blood or urine samples based on the formation of ¹⁴CO₂ from the metabolism of [¹⁴C]glucose (1, 5). Because many current gas-measuring methods are rather elaborate, interest has centered around the development of simple methods such as a syringe to collect the gas produced by rumen microorganisms (2, 3, 6) or by submerging a sealed bag, containing inoculated fruit, in water and measuring the gas volume by the displaced water volume (4). Apart from the questionable accuracy in measuring gas pressure by these methods, the need to manually record the data is an obvious disadvantage.

This report describes a method for measuring gas production by using a pressure transducer to sense gas pressure buildup. The equipment is easy to assemble and use and has the added advantage that the rate of gas produced can be measured automatically and accurately. The transducer method was used to study gas production by members of the coliform group of bacteria.

MATERIALS AND METHODS

Cultures. The following cultures were obtained from the American Type Culture Collection (Rockville, Md.): *Escherichia coli* 12014, *Enterobacter aerogenes* 13882, and *Citrobacter intermedium* 6750. Cultures were maintained at 5 C on Trypticase soy agar slants (TSA, BBL) and transferred monthly.

Inoculum preparation, viable counts, and media. Inocula for the gas pressure measurements were prepared by making 10-fold dilutions of a 24-h Trypticase soy broth culture (TSB, BBL) in sterile 0.05% peptone broth and by adding 1 ml of appropriate dilutions to 9 ml of media pre-warmed to experimental conditions. Viable counts were made by spreading appropriate dilutions from the 10-fold series on TSA and counting colonies after 24 h of incubation at 35 C. Lauryl tryptose broth (LTB, Difco), Eijkman lactose medium (BBL), phenol red broth base with 0.5% lactose (Difco), and brilliant green bile broth (BBL) were used in this study.

Gas pressure measurements. The basic components of the pressure transducer measurement system are shown in Fig. 1. A metal cap, machined to fit a test tube (20 by 150 mm), was drilled to accept a 5.0-lb/in² strain gauge transducer (Stratham Instruments, Inc., Oxnard, Calif.) and a 20-gauge needle. The inlet tube of the transducer and the needle were inserted through the cap and soldered in place; a

silicone rubber gasket was positioned inside the cap to form a gas-tight seal. Attached to the needle was a Luer-Lok one-way stopcock to equalize pressure inside the test tube after the cap was positioned in place. Sterilization of the pressure transducer assembly was accomplished by exposing the interior, threaded portion of the metal cap to two ultraviolet lamps (G15T8, General Electric, Richmond Heights, Ohio) for 30 min. The culture tube-transducer assembly was placed in an incubator, a water bath, or was shaken at 200 oscillations/min on a reciprocal shaker in a water bath (Precision Scientific Co., Chicago, Ill.). The electrical output of the transducer was

connected to a DC power supply and a strip-chart recorder (model 194, Honeywell Industrial Div., Fort Washington, Pa.), and all measurements on the recorder were made on the 50-mV scale. In addition to recorder monitoring, an AC-DC digital-reading voltmeter (Ballentine Labs., Boonton, N.J.) was used to read mV response directly. Samples for CO_2 analysis were obtained by attaching a syringe to the pressure equalizer valve and by removing 2 ml of the headspace gas. The CO_2 measurements were done on an F & M 810 gas chromatograph (Hewlett-Packard, Avondale, Pa.) equipped with a thermal conductivity detector and a 0.25-in (6.35-mm outside diameter) by 12-ft (3.66 m) stainless steel column packed with Porapak Q.

RESULTS AND DISCUSSION

Effect of inoculum size. A strip-chart tracing of gas pressure response curves for various inocula of *E. aerogenes* is shown in Fig. 2. After about 1 h of incubation, the initial pressure increased because of tightening of the cap and heating of the compressed internal gases and was released by opening the stopcock and then closing it to establish a zero base line. Characteristically, the gas pressure response curves consisted of a lag period with no increase in pressure, followed by a period of rapid buildup in pressure and then a leveling-off period (Fig. 3). The linear relationship between inoculum size and length of the lag period for *E. coli*, *E. aerogenes*, and *C. intermedium*, tested in LTB at 35 C, is shown in Fig. 4. These data are summarized in Table 1, which shows the standard error of the mean for the four levels of inocula tested. Because no differences were noted in length of the lag period for stationary cultures tested in a water bath or incubator or for cultures shaken at 200 oscillations/min, these data were pooled in Table 1 and Fig. 4. Lag times ranged from 3 h for 10^7 cells/ml to 12 h for 10^0 cells/ml. Studies with a wide range of

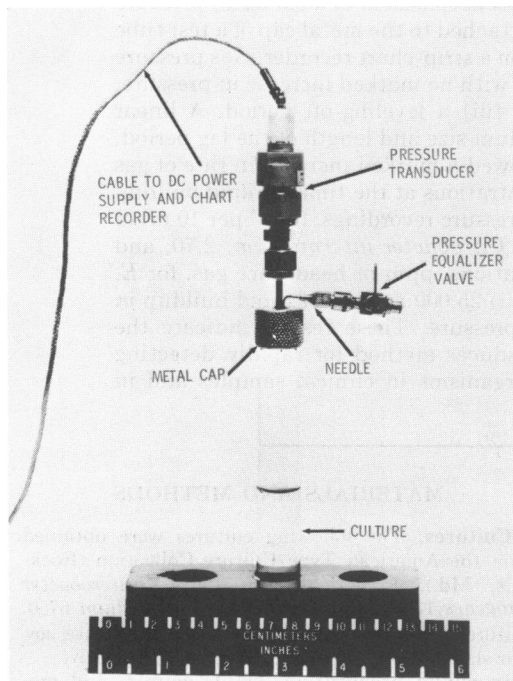


FIG. 1. Major components of the pressure transducer system for measuring gas production.

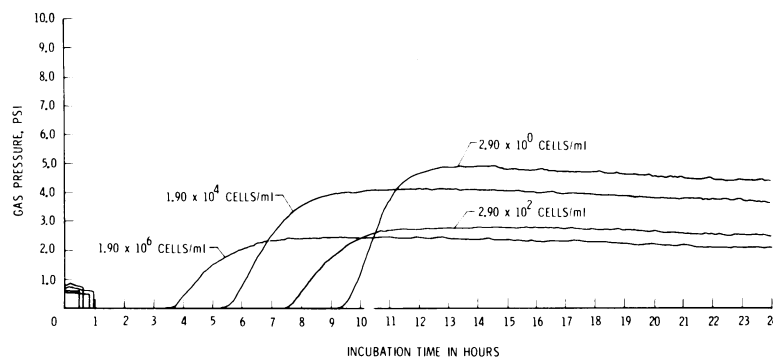


FIG. 2. Strip-chart tracing showing variations with time of gas pressure responses for various inocula of *Enterobacter aerogenes*. Cultures were shaken at 200 oscillations/min at 40 C in 10 ml of lauryl tryptose broth.

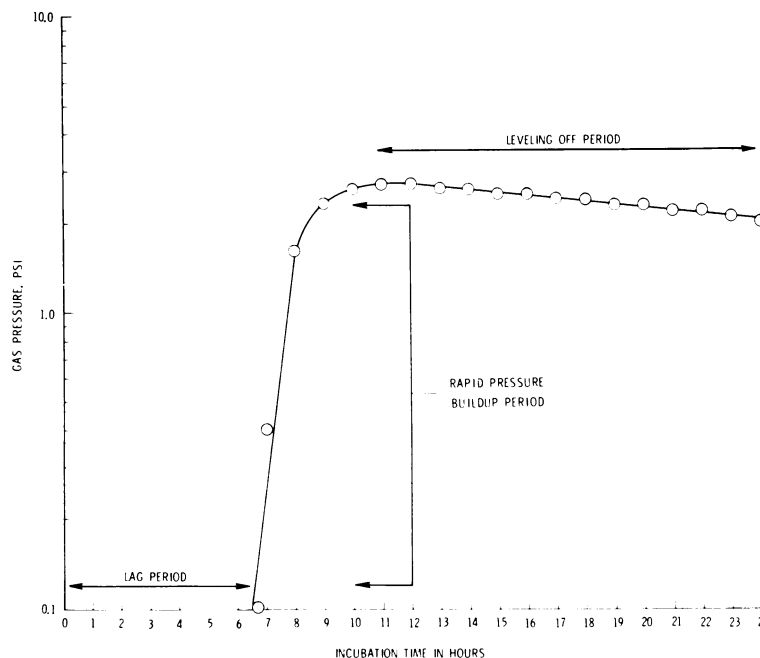


FIG. 3. Typical gas pressure curve depicting the periods used for interpreting gas pressure response curves. *Escherichia coli* shaken at 200 oscillations/min in 10 ml of lauryl tryptose broth at 35 C; inoculum was 1.16×10^4 cells/ml.

inoculum levels indicated that each 10-fold increment of cells reduced lag time by 60 to 70 min. Because initial tests showed no differences in the response curves for washed or unwashed cells, these studies were conducted with unwashed cells.

Effect of incubation conditions. Various incubation conditions markedly altered the shape of the gas pressure response curves (Fig. 5). When stationary cultures were tested in a water bath or incubator, the slope of the rapid buildup in the pressure period was gradual with a steady increase in pressure. On the other hand, when cultures were shaken at 200 oscillations/min in a water bath, the slope of the rapid buildup in the pressure curve was steep, with maximum pressure responses occurring in about 4 to 5 h. Shaken cultures showed a loss of pressure after about 3 to 4 h in the leveling-off period, with a continual gradual decline, and represented a total loss of about 0.5 to 1.0 lb/in² in 24 h. The maximum amount of gas pressure recorded depended on incubation conditions and cultures. For all test conditions, *E. aerogenes* exhibited the highest pressure recordings with a mean of 3.70 lb/in² per 10 ml of broth, followed by *C. intermedium* and *E. coli* with measurements of 2.70 and 2.10 lb/in² per 10 ml of broth, respectively. In general, the

highest values for the rate of pressure increase and for maximum pressure recordings (lb/in² per 10 ml of broth) were, in order of decreasing intensities, (i) shaken water bath, (ii) stationary water bath, and (iii) stationary incubator conditions.

Effect of temperature. When the three species were tested in 10 ml of LTB and shaken at 200 oscillations/min, incubation at 30, 35, or 40 C did not markedly influence the shape of the gas pressure response curves, but it did effect length of the lag period (Fig. 6). Incubation at 40 C reduced lag times for *E. coli* and *E. aerogenes* by 1 to 2 h as compared with incubation at 35 C, but no differences were noted for *C. intermedium* at these temperatures. For the three species tested, incubation at 30 C increased lag times by 2 to 7 h over 35 and 40 C, respectively. At 45 C, *E. aerogenes* and *C. intermedium* failed to grow in 24 h at the inoculum levels tested. *E. coli* lag periods at 45 C for 10^6 and 10^4 cells/ml were 9 h and 30 min and 17 h and 40 min, respectively, an increase of 5 to 11 h over incubation at 35 and 40 C. At concentrations of 10^2 and 10^0 cells/ml, no growth of *E. coli* was evident in 24 h at 45 C.

Cell numbers and CO₂ concentrations at times of rapid buildup in pressure. The number of cells present in 10 ml of LTB shaken at

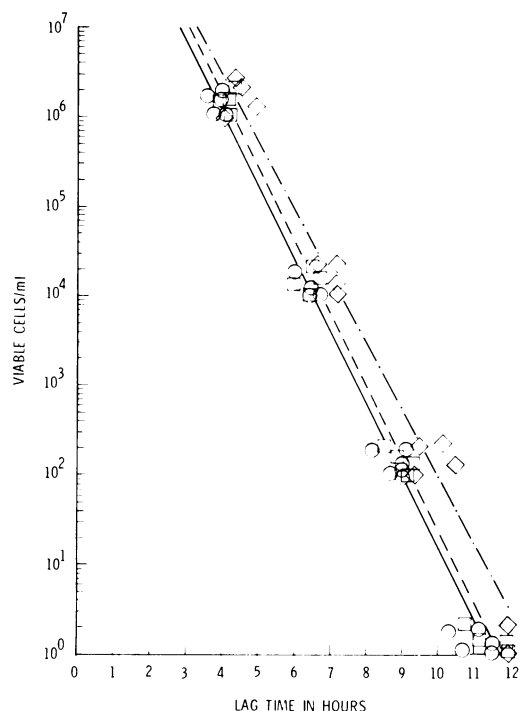


FIG. 4. Relationship between inoculum size and length of lag period. Symbols: \circ , *Escherichia coli*; \square , *Enterobacter aerogenes*; and \diamond , *Citrobacter inter-*

medium. Lines were fitted by the method of least squares; correlation coefficients were *E. coli* 0.9898, *E. aerogenes* 0.9224, and *C. intermedium* 0.9886. For an explanation of pooled data, see text.

TABLE 1. Effect of inoculum level on length of lag period^a

Inoculum (cells/ml)	<i>Escherichia coli</i>	<i>Enterobacter aerogenes</i>	<i>Citrobacter intermedium</i>
10^6	238 ± 4.98^b	254 ± 6.16	272 ± 9.62
10^4	382 ± 7.35	390 ± 3.77	411 ± 10.15
10^2	520 ± 3.61	518 ± 6.75	580 ± 17.36
10^0	677 ± 12.37	669 ± 18.37	718 ± 15.66

^a Pooled data, see text for details; 10 ml of lauryl tryptose broth, 35 C.

^b Lag time in minutes \pm standard error of the mean.

^c Results of 5 to 10 separate determinations.

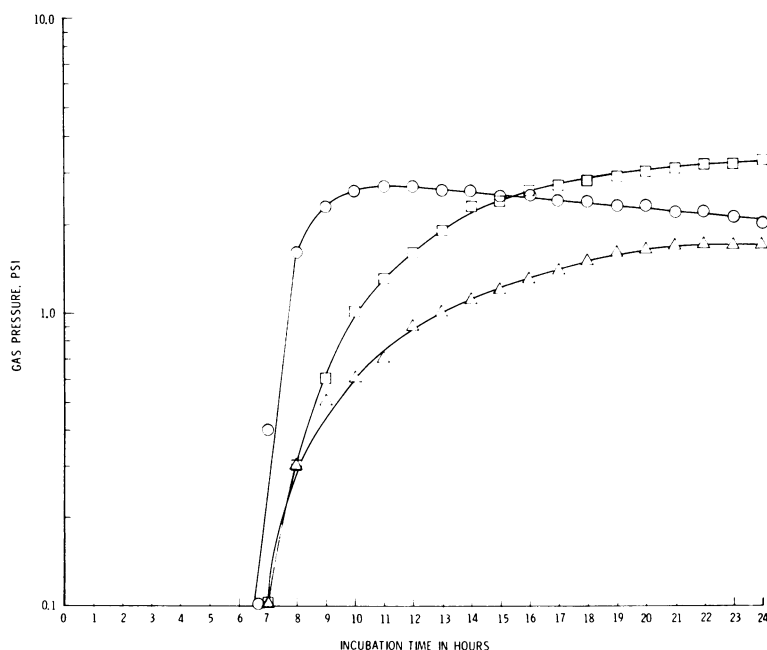


FIG. 5. Effect of incubation conditions on gas pressure response curves with *Escherichia coli* at 35 C in 10 ml of lauryl tryptose broth. Symbols: \circ , shaken at 200 oscillations/min in a water bath; \square , static culture in a water bath; and Δ , static culture in an incubator. Inoculum size ranged from 1.08×10^4 to 1.83×10^4 cells/ml.

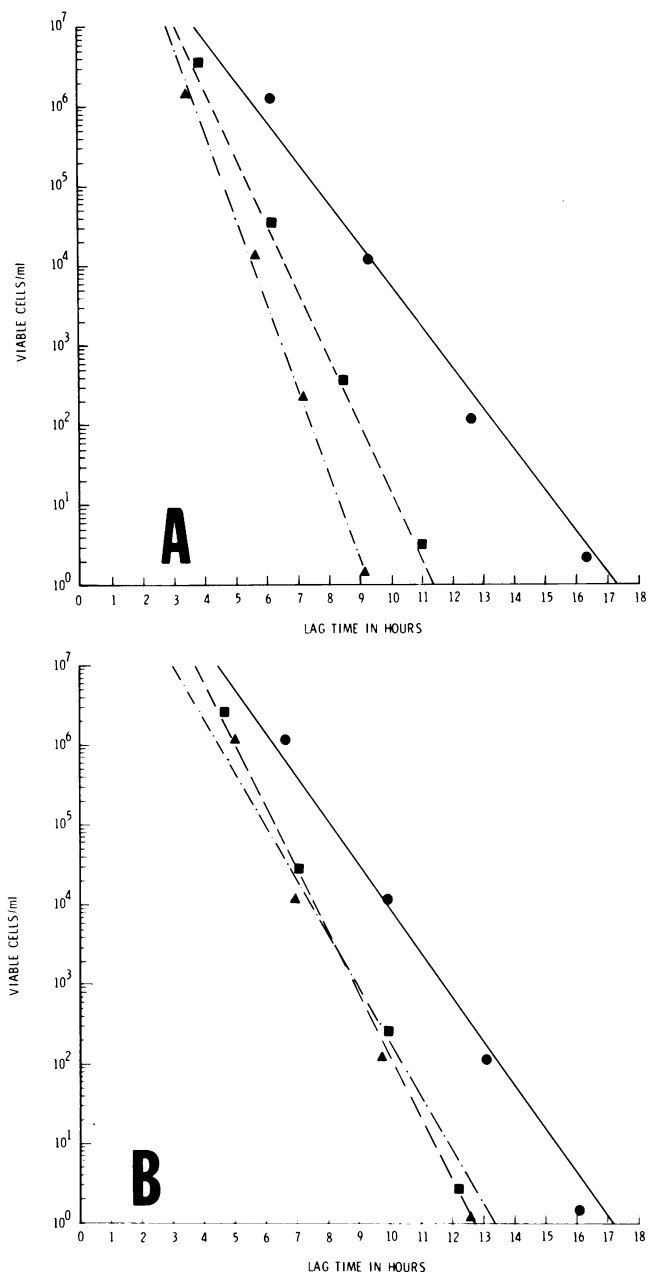


FIG. 6. Effect of temperature on length of lag period for (A) *Escherichia coli* and (B) *Citrobacter intermedium*. Cultures were shaken at 200 oscillations/min in a water bath (10 ml lauryl tryptose broth). Symbols: ●, 30 C; ■, 35 C; and ▲, 40 C. Each point represents the mean of three separate determinations. Lines were fitted by the method of least squares; correlation coefficients ranged from 0.9374 to 0.9989.

TABLE 2. Cell numbers and CO₂ concentration at time of rapid buildup in pressure

Culture ^a	No. of cells/ ml in 10 ml of LTB	CO ₂ con- centration (ppm)
<i>Escherichia coli</i>	7.75×10^{8b}	27,075 ^b
<i>Enterobacter aerogenes</i>	5.40×10^8	24,870
<i>Citrobacter intermedium</i>	6.55×10^8	23,000

^a Cultures were shaken at 200 oscillations/min at 35 C.

^b Means of four separate determinations.

25,000 at pressure buildup, and 150,000 at time of maximum pressure reading. The interesting relationships between gas pressure, cell numbers, and CO₂ concentrations, especially at the time of rapid buildup in pressure, are currently under investigation.

The data reported here suggest several practical applications of the pressure transducer system for detecting gas-producing microorganisms. The first obvious application is early detection of coliforms in water samples; the device is also applicable to other problems in sanitary microbiology. Clinical and food microbiologists might use the pressure transducer system for detection of pathogens and in steril-

ity and quality control determinations; microbial physiologists could adapt it to Warburg respirometry. The pressure transducer system could be used in any application where gas production by microorganisms is measured.

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